Glutathione *S*-Transferase Class μ Deletion Polymorphism and Breast Cancer: Results from Prevalent *versus* Incident Cases[1]

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Abstract

A common deletion polymorphism in the gene coding for the glutathione *S*-transferase class μ (the *GSTM1* gene) results in a decreased ability to detoxify carcinogenic epoxide intermediates and has been associated with increased breast cancer risk in some small studies. We studied the *GSTM1* gene deletion polymorphism (conferring the null genotype) in 245 women who had prevalent breast cancer and 245 women without breast cancer, who were among the 32,826 women in the Nurses' Health Study who gave a blood sample in 1989–1990. In the prevalent case series, the null genotype was slightly more common among cases (58%) than among controls (51%; age-adjusted odds ratio = 1.30; 95% confidence interval, 0.91–1.86). Among cases, the prevalence of the *GSTM1* deletion increased with duration of survival [68% for ≥8 years since diagnosis; 57% for 4–8 years; 51% for <4 years; *P* (trend) = 0.04]. In an incident case series of 240 women who were diagnosed with breast cancer following blood collection and prior to June of 1992 and compared with age-matched controls, the *GSTM1* deletion was not associated with an elevation in risk (relative risk, 1.08; 95% confidence interval, 0.74–1.57). No significant interaction with cigarette smoking was evident. Thus, there was no significant increase in risk of incident breast cancer associated with the *GSTM1* null genotype; however, the gene deletion polymorphism appeared to confer improved survival. These data suggest that odds ratios based upon prevalent cases in molecular epidemiologic studies may be biased due to differential survival. Further studies are required to determine whether this polymorphism is associated with improved breast cancer prognosis.

Introduction

The incidence of breast cancer has increased approximately 1% annually over the past 50 years, and breast cancer currently accounts for about one-third of the incident cancers among women who live in the United States (1). A substantial portion of this increase has been attributed to changes in the known risk factors of age at menarche, age at first birth, and parity (2–4). However, some recent studies have reported that environmental and occupational factors are associated with breast cancer occurrence, leading to the suggestion that certain environmental factors may also be contributing to the increasing incidence of breast cancer (5).

Prominent among such factors are PAHs.[3] Exposure to this class of compounds is ubiquitous in modern life; they are human and animal carcinogens (6), they induce mammary tumors in animal models (particularly if exposure occurs prior to first pregnancy; Ref. 7), and they induce malignant transformation of breast epithelial cells (8). Several laboratories have studied the possible role of PAH exposure in mammary tumorigenesis using ^{32}P postlabeling to detect aromatic DNA adducts in the target breast tissue. Seidman *et al.* (9) reported detectable aromatic adducts in 3 of 10 reduction mammoplasty samples. Routledge *et al.* (10) found similar adducts in 5 of 24 autopsy breast samples. Perera *et al.* (11) examined normal tissue adjacent to tumors in 15 breast cancer patients and normal tissue from 4 patients undergoing reduction mammoplasty and observed aromatic adducts in tissue from all patients, with smoking-related damage evident in 5 of the 15 cases. Finally, Li *et al.* (12) compared DNA adducts in normal breast tissue from surgical specimens of 87 breast cancer patients and in normal tissue from 29 patients undergoing reduction mammoplasty. Aromatic adducts were found in all tissues examined, and the breast cancer patients had significantly higher levels of adducts than controls.

Most polyaromatic compounds are metabolically activated by the family of cytochrome P-450 enzymes to generate reactive epoxide intermediates. These epoxides are then further metabolically conjugated to water-soluble intermediates in further detoxication steps. Genetic variation in these pathways has been widely studied, including a now well-described deletion polymorphism in the GST class μ. The GSTs [EC 2.5.1.18] are a family of enzymes that detoxify reactive electrophiles, such as epoxides, which can act as mutagens (13). There are several

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[3] The abbreviations used are: PAH, polyaromatic hydrocarbon; GST, glutathione *S*-transferase; OR, odds ratio; CI, confidence interval; LRT, likelihood ratio test.

classes of GSTs found in humans, including the μ, π, θ, and α classes. Phenotypic activity of the GST μ enzyme has been found to be highly polymorphic in the population (14). The variation in conjugation of epoxide substrate intermediates has been observed to segregate with inherited loss of the *GSTM1* gene; that is, individuals who inherit the homozygous form of the null polymorphism in the *GSTM1* gene are not capable of conjugating and detoxifying specific substrate epoxide intermediates (15).

There has been considerable recent interest in the possible association of this null genotype with susceptibility to exposure-induced malignant disease. The known substrates for the GSTM1 protein product include PAHs, specifically including metabolically generated epoxide intermediates of benzo(α)pyrene; several groups have observed positive associations of the *GSTM1*-null genotype and smoking-induced lung (reviewed in Ref. 16) and bladder cancer (reviewed in Ref. 17). In addition, several laboratories have also examined the prevalence of the null genotype in case-control studies of breast cancer (18–20). However, these studies were relatively small, and none used a prospective design. Zhong *et al.* (18) compared the prevalence of the null genotype in patients with breast cancer and controls and observed a nonsignificant excess of GSTM1 deletion in the breast cancer cases (OR = 1.27). Ambrosone *et al.* (19) studied 212 postmenopausal caucasian women and 282 community controls, and reported that the GSTM1 deletion was not associated with breast cancer risk. However, their data do suggest an increase in risk among the youngest postmenopausal women (OR = 2.44). Paradiso *et al.* (20) found no difference in the prevalence of the deleted phenotype among 63 breast cancer patients and 45 healthy controls, but they did note that tumor ploidy was associated with the GST μ phenotype.

In addition, because breast cancer has been treated using a wide variety of alkylating chemotherapeutic agents that may act as substrates for the GSTM1 protein product, there has also been substantial interest in the possible role of this and other classes of GSTs in conjugation with the bioactive compounds given to patients in treatment for their disease, potentially reducing the effectiveness of these agents as cytotoxins and leading to the subsequent failure of cancer treatment. A number of small studies (primarily case series, cross-sectional in design) having suggested that GST π expression may be associated with the occurrence of invasive ductal carcinomas (21) and with estrogen receptor status (22). Others have reported that the GST μ deletion may be associated with higher-grade tumors (23). At the same time, several studies have not demonstrated any association of GST expression with sensitivity to chemotherapeutic drugs or with outcome of treatment for breast cancer (24, 25).

To determine if the *GSTM1* deletion polymorphism is associated with risk of breast cancer or with length of survival, we have studied the *GSTM1* gene deletion polymorphism in 245 women who had prevalent breast cancer and 245 women without breast cancer, who were among the 32,826 women in the Nurses' Health Study who gave a blood sample in 1989–1990; we also studied 240 incident cases diagnosed after giving blood sample and 240 controls.

Subjects and Methods

Study Population. In 1976, 121,700 married registered nurses from 11 states were enrolled in the Nurses' Health Study and have been subsequently followed by questionnaire every 2 years. Self-reported diagnoses of breast cancer are confirmed by medical record review (26), and follow-up through 1992 is greater than 95% of potential person-years. Information on risk factors previously associated with breast cancer, including family and reproductive history, is obtained by questionnaire and updated periodically. Age of onset of smoking and the number of cigarettes smoked per day during early life was ascertained on the baseline questionnaire in 1976, as was the age of quitting for past smokers. Subsequent to 1976, the number of cigarettes smoked per day has been ascertained every 2 years.

In 1989–1990, 32,826 women provided blood samples that were separated into plasma, erythrocyte, and buffy coat components and stored in liquid nitrogen freezers. For the current study, prevalent cases were defined as women who had a confirmed diagnosis of breast cancer at the time the blood sample was provided. We selected 245 cases who had been diagnosed with breast cancer between enrollment in the study in 1976 and the date they returned a blood sample; in this group, we oversampled cases with a positive family history by including all women with a history of breast cancer in their mother (n = 92), their sister (n = 56), or both their mother and sister (n = 12); the remaining 85 were selected at random from among the approximately 800 prevalent cases with no family history of this disease. Oversampling was performed to enrich the pool of subjects in which a genetic factor might play an etiologic role in the disease occurrence. Controls were free of diagnosed cancer (other than nonmelanoma skin cancer) at the time they gave a blood sample; they were matched to the cases on year of birth and were otherwise randomly selected. Incident cases were defined as women who did not have a diagnosis of cancer (other than nonmelanoma skin cancer) when they provided the blood sample but were subsequently diagnosed with breast cancer prior to June 1, 1992; 240 eligible incident cases were identified (198 invasive, 39 *in situ*, and 3 of uncertain invasiveness). For each incident case, we randomly sampled one control matched on year of birth, menopausal status, month of blood return, time of day of blood draw, overnight fasting status, and postmenopausal hormone use. Both cases and controls were >95% caucasian.

Statistical Analysis. We calculated ORs and 95% CIs for the association of the *GSTM1* null genotype with breast cancer using conditional logistic regression. Estimates of the interaction between smoking and genotype were calculated by including indicator variables for each category of smoking exposure for each genotype in multivariate models; the hypothesized low-risk category (i.e., *GSTM1* nondeleted, nonsmokers) served as the referent category for the model. Statistical significance was assessed by using a LRT to compare the goodness of fit of the model with these interaction terms, with the reduced model containing indicator variables for the main effects of genotype and exposure (*i.e.*, without interaction terms), and with potentially confounding variables, such as menopausal hormone use (primarily estrogen replacement therapy).

Laboratory Methods. Genotyping for the *GSTM1* deletion was completed using PCR-based methods published previously (27). Laboratory personnel were blind to case-control status, and multiple repeat samples were included in the PCR analysis to monitor quality control; all repeat samples were concordant. The genotype for the *GSTM1* deletion has been demonstrated previously to reflect phenotype in essentially all cases (28).

Results

In the prevalent case-control analysis, 58% of the cases were homozygous-deleted in the *GSTM1* gene, compared to 51% of

Table 1 *GSTM1* deletion polymorphism and breast cancer in prevalent cases and age-matched controls[a]

	GSTM1 gene[b]	
	Deleted	Present
Prevalent cases	141 (58%)	103 (42%)
Controls	126 (51%)	119 (49%)

[a] OR was conditional on age, menopausal status, postmenopausal hormone use, overnight fasting status, and month of blood return; OR = 1.30 (95% CI, 0.91–1.86).
[b] One case sample was not genotyped.

Table 2 Years since breast cancer diagnosis in prevalent cases stratified by GST μ deletion status

	Years since diagnosis[a]		
	<4 yr	4–8 yr	>8 yr
GSTM1-null	44 (51%)[b]	52 (57%)	44 (68%)
GSTM1-positive	43 (49%)	39 (43%)	21 (32%)

[a] Years since diagnosis was not known for one individual, and one sample was not genotyped.
[b] Percent of the total *GSTM1*-null and *GSTM1*-positive individuals in each stratum; $P_{trend} = 0.04$.

Table 3 The association of the *GSTM1* gene deletion and incident breast cancer[a]

	GSTM1 gene	
	Deleted	Present
Cases	119 (50%)	121 (50%)
Controls[b]	115 (48%)	124 (52%)

[a] OR matched on age, menopausal status, postmenopausal hormone use, and month of blood return was 1.08 (95% CI, 0.74–1.57); OR additionally adjusted for family history of breast cancer, age at first birth, history of bening breast disease, and body mass index was 1.06 (95% CI, 0.71–1.58).
[b] One control sample was not genotyped.

Table 4 ORs and 95% CIs for breast cancer risk stratified by *GSTM1* genotype and years smoked prior to first pregnancy[a]

	Years smoked		
	Never	0–5 yr	5+ yr
GSTM1-positive			
Cases	57	19	38
Controls	51	16	36
Matched OR[b]	1.0 (ref.)[d]	1.14 (0.51–2.58)	0.91 (0.48–1.73)
Adjusted OR[c]	1.0 (ref.)	1.21 (0.49–3.03)	0.76 (0.37–1.55)
GSTM1-null			
Cases	37	25	43
Controls	49	21	30
Matched OR[b]	0.74 (0.39–1.40)	0.94 (0.46–1.94)	1.35 (0.71–2.56)
Adjusted OR[c]	0.64 (0.31–1.25)	0.98 (0.43–2.16)	1.34 (0.64–2.61)

[a] Nulliparous subjects were excluded. LRT for interaction: $\chi^2 = 3.40$; $P = 0.18$; 2 degrees of freedom.
[b] OR was matched on age, menopausal status, postmenopausal hormone use, and month of blood return.
[c] OR was additionally adjusted for family history of breast cancer, age at firth birth, history of benign breast disease, and body mass index.
[d] Referent.

Table 5 ORs and 95% CIs for breast cancer risk stratified by *GSTM1* genotype and smoking status shortly prior to diagnosis[a]

	Smoking status		
	Never	Past	Current
GSTM1-positive			
Cases	59	50	12
Controls	57	55	12
Matched OR[b]	1.0 (ref.)[d]	0.86 (0.51–1.45)	0.97 (0.38–2.47)
Adjusted OR[c]	1.0 (ref.)	0.87 (0.49–1.56)	1.01 (0.35–2.91)
GSTM1-null			
Cases	44	57	18
Controls	53	50	12
Matched OR[b]	0.78 (0.44–1.38)	1.10 (0.65–1.85)	1.52 (0.68–3.37)
Adjusted OR[c]	0.71 (0.38–1.32)	1.10 (0.62–1.94)	1.89 (0.78–4.57)

[a] LRT for interaction: $\chi^2 = 2.53$; $P = 0.28$; 2 degrees of freedom.
[b] OR was matched on age, menopausal status, postmenopausal hormone use, and month of blood return.
[c] OR was additionally adjusted for family history of breast cancer, age at firth birth, history of benign breast disease, and body mass index.
[d] Referent.

the controls (Table 1). The prevalence of the *GSTM1* deletion polymorphism was almost identical among cases with a mother or sister history of breast cancer (59%) and among cases without this family history (58%). The OR of 1.30 (95% CI, 0.91–1.86) for the *GSTM1* deletion polymorphism was not statistically significant. When we restricted the analysis to women without a family history, the OR was 1.18 (95% CI, 0.73–1.92). However, when we examined the distribution of cases stratified by years since diagnosis, there was a significant trend toward an increase in the prevalence of the polymorphism with increasing time since diagnosis of disease ($P_{trend} = 0.04$; Table 2).

When we studied incident cases, 50% were *GSTM1*-deleted (119 of 240), compared with 48% of the controls (115 of 239; one control sample did not amplify in PCR). The matched OR was 1.08 (95% CI, 0.74–1.57); the multivariate-adjusted conditional OR (adjusted for possible confounders, including family history of breast cancer, age at first birth, history of benign breast disease, and body mass index) was 1.06 (95% CI, 0.71–1.58; Table 3).

Because other data have suggested that there is an increased risk of breast cancer associated with smoking, particularly at a young age, that may be modified by genetic differences in metabolic capacity (18), we examined the incident case-control data for an interaction between smoking and *GSTM1* genotype. There was no significant crude or adjusted interaction between the null polymorphism and smoking before first pregnancy. When we further stratified by years smoked before pregnancy, again there was no significant interaction (Table 4). When smoking status was defined shortly prior to diagnosis in the cases, current smokers who were *GSTM1*-null were at nonsignificantly higher risk than the *GSTM1*-positive nonsmokers; no significant interaction was present (Table 5). Again, there was no evidence of an interaction with recent smoking status when a continuous cigarette dose term was included (data not shown). Finally, we performed similar analyses for interactions between smoking and *GSTM1* status using smoking status 10 years prior to diagnosis, smoking status at the time of subject enrollment, and using total pack-years of smoking. Again, no significant interaction was observed (data not shown).

We also examined the data for effect modification by menopausal status. There were 105 women that were premenopausal, and 326 who were postmenopausal. The matched OR for the premenopausal analysis was 0.83 (95% CI, 0.25–2.73). The matched OR for the postmenopausal analysis was 1.11

(95% CI, 0.71–1.76). The multivariate adjusted analysis did not differ significantly from the matched analysis in either case.

Discussion

The deletion polymorphism in glutathione *S*-transferase class μ was not associated with incident breast cancer in this study. The point estimated for the adjusted OR was 1.06, with an upper confidence limit of 1.58. This result is consistent with previous case-control investigations that also found no significant association or only a weak association of the *GSTM1* deletion and breast cancer.

We also found no significant association between smoking and breast cancer, with no significant interaction with *GSTM1* genotype when it was stratified by smoking before first pregnancy, smoking 10 years prior to diagnosis, or smoking shortly prior to diagnosis. The point estimate for the OR in the highest smoking exposure category was consistently elevated, but it did not reach significance in any analysis. Thus, it is unlikely that the *GSTM1* deletion is a major contributor to breast cancer risk among smokers or nonsmokers. Additional years of follow-up will be needed to determine if a modest association exists.

The lack of an association between the *GSTM1* deletion and breast cancer risk is interesting, in light of the recent studies that have reported significantly elevated levels of polyaromatic DNA adducts in breast tissue from cancer patients (9–12). Other studies have associated the *GSTM1* deletion polymorphism with susceptibility to the formation of polyaromatic DNA adducts in lungs of smokers (29, 30). Thus, it would be of interest to know if the *GSTM1* deletion contributes to enhanced formation of PAH DNA adducts in the breast. Li *et al.* (12) have asserted that the spectrum of the PAH adducts seen in breast tissue is different from that reported for lung tissue, and they further postulated that the source of the PAH may not be cigarettes. Our finding of no association of the null genotype of *GSTM1* with breast cancer, combined with this hypothesis, would predict that the null polymorphism is not associated with the enhanced formation of these DNA adducts in the breast.

Interestingly, we found that the GST μ polymorphism was significantly associated with years since diagnosis in the prevalent cases, suggesting that it may offer a survival advantage among cases. The mechanism responsible for improved survival could be related to a better response to alkylating chemotherapeutic agents or radiation used to treat this disease; unfortunately, we did not have detailed information on previous treatment of the cases we studied. Among the most active chemotherapeutic agents in the treatment of breast cancer are cyclophosphamide and thiotepa, as well as anthracyclines, such as doxorubicin. These compounds are all conjugated with thiols through reactions mediated by GSTs (31). The π class of transferases is thought to be the major route of conjugation and subsequent prevention of DNA alkylation (31), although there has been some suggestion that the μ class is also involved (32). The regulation and induction of the transferases are not thoroughly understood, and there is some evidence that individuals who lack the *GSTM1* gene have differing patterns of enzyme induction (33). This could extend to other classes of GSTs, although we are not aware of any data addressing this possibility. If the GST class μ is important in protecting cells from high doses of chemotherapeutics, patients who have the null polymorphism might be expected to have more effective cell killing by the treatment, leading to better survival. It is also possible that the GST class μ acts to protect cells from radiation-induced damage, as it has been demonstrated that glutathione is a radioprotector (34). This is consistent with the demonstration that *GSTM1*-deleted individuals are susceptible to asbestos-induced interstitial lung disease, also thought to be caused by a similar oxidative mechanism (35). A cross-sectional study such as ours, with prevalent cases that were oversampled for a family history of breast cancer, is clearly not the optimal way to examine this association. Retrospective or prospective studies of women treated for breast cancer with detailed information of the type and timing of treatment are needed.

It is also important to note that oversampling of prevalent cases with a family history of breast cancer could also bias our result. It is possible that there could be confounding or effect modification of the associations between breast cancer survival, family history of breast cancer, and *GSTM1* genotype. Breast cancer that has an inherited component may occur in an etiologically distinct fashion from that of truly sporadic disease. Hence, our observation of an association of breast cancer survival with the *GSTM1* genotype may not generalizable to all women with this disease.

Finally, our results also indicate how the inclusion of prevalent cases in case-control studies of traits that may confer a survival advantage might be biased. ORs based upon prevalent cases can be biased estimates of the relative risk for incident disease due to a "survivor effect." This must be considered in the study of diseases in which the survival or therapeutic outcome can vary.

Regardless of the mechanism, our data suggest that the *GSTM1* deletion polymorphism is not appreciably associated with the occurrence of breast cancer; the possibility of an association with survival after treatment deserves further study.

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